

FORM PTO-1390

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY DOCKET NUMBER **WARF-0174**

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

U.S. APPLICATION NO. (if known see 37 C.F.R. 1.5)

10/009097INTERNATIONAL APPLICATION NO.
PCT/US00/15693INTERNATIONAL FILING DATE
08 JUNE 2000 (08.06.00)PRIORITY DATE CLAIMED **09 JUNE 1999**
(09.06.99)TITLE OF INVENTION **UNFERMENTED GEL FRACTION FROM PSYLLIUM SEED HUSKS**APPLICANT(S) FOR DO/EO/US **MARLETT, Judith A.; FISCHER, Milton H.**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ has been transmitted by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US)
 - d. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
6. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
7. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
8. ☐ An oath or declaration of the inventor(s) 35 U.S.C. 371(c)(4).
9. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☒ A change of power of attorney and/or address letter.
17. ☒ Other items or information: **Copy of Publication WO 00/74689 dated 14 December 2000 including search report; Copy of International Preliminary Examination Report; return postcard; check in amount of \$836.00**

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the U.S. Patent and Trademark Office, Arlington, VA 22202

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Justin Haskowski
Justin Haskowski

U.S. APPLICATION NO. **10/009097**INTERNATIONAL APPLICATION NO.
PCT/US00/15693ATTORNEY DOCKET NUMBER
WARF-017417. ☒ The following fees are submitted:**Basic National Fee (37 CFR 1.492(a)(1) - (5)):**Neither international preliminary examination fee (37 CFR 1.482)
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO
and International Search Report not prepared by the EPO or JPO.....\$1,040.00International preliminary examination fee (37 CFR 1.482 not paid to USPTO
but International Search Report has been prepared by the EPO or JPO.....\$890.00International preliminary examination fee (37 CFR 1.482) not paid to USPTO but
international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$740.00International preliminary examination fee paid to USPTO (37 CFR 1.482) but
all claims did not satisfy provisions of PCT Article 33(1)-(4).....\$710.00International preliminary examination fee paid to USPTO (37 CFR 1.482) and
all claims satisfied provisions of PCT Article 33(1)-(4).....\$100.00**ENTER APPROPRIATE BASIC FEE AMOUNT =** \$710.00Surcharge of \$130.00 for furnishing the oath or declaration later than _ 20 _ 30 months from
the earliest claimed priority date (37 CFR 1.492(e)). \$

Claims	Number Filed	Number Extra	Rate		
Total claims	27 - 20 =	7	X \$18.00	\$126.00	
Independent Claims	3 - 3 =	0	x \$84.00	\$---	
Multiple dependent claims(s) (if applicable)			+ \$280.00	\$--	

TOTAL OF ABOVE CALCULATIONS = \$836.00Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are
reduced by 1/2. \$**SUBTOTAL =** \$836.00Processing fee of \$130.00 for furnishing the English translation later than _ 20 _ 30 months
from the earliest claimed priority date (37 CFR 1.492(f)). + \$**TOTAL NATIONAL FEE =** \$836.00Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +**TOTAL FEES ENCLOSED =** \$836.00Amount to be:
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a. ☒ A check in the amount of \$ **836.00** to cover the above fee is enclosed.b. ☐ Please charge my Deposit Account No. 23-3050 in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account
No. 23-3050. A duplicate copy of this sheet is enclosed.**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must
be filed and granted to restore the application to pending status.**

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REGISTRATION NUMBER

**UNFERMENTED GEL FRACTION FROM
PSYLLIUM SEED HUSKS**

Pursuant to 35 U.S.C. §202(c), it is
acknowledged that the U.S. Government has certain rights
in the invention described herein, which was made in part
with funds from the National Institutes of Health, Grant
5 No. DK21712.

This application claims priority to U.S.
Application No. 09/328,611, filed June 9, 1999, the
entirety of which is incorporated by reference herein.

10 FIELD OF THE INVENTION

This invention relates to the field of
laxatives, treatments to lower blood serum cholesterol
and low-calorie food thickeners and fat substitutes. In
particular, the invention relates to unfermented gel-
15 forming polysaccharides from psyllium seed husks and
methods for their isolation.

BACKGROUND OF THE INVENTION

Various scientific and scholarly articles are
20 referred to in parentheses throughout the specification.
These articles are incorporated by reference herein to
describe the state of the art to which this invention
pertains.

The seed husks of psyllium (*Plantago ovata*,
25 also known as ispaghula) are commonly used as a laxative
and to promote regular bowel function. Psyllium seed
husks promote laxation partially by increasing the mass
and moisture content of the stool (Marteau et al., 1994,
Gut 35:1747-1752). Additionally, the excreta of animals
30 and humans fed diets containing psyllium seed husk is

gelatinous. This gelatinous property contributes to laxative properties of psyllium seed husks by decreasing friction in the gut. Observed increases in fecal mass and water retention also have been attributed to this gelatinous material (Marteau et al., 1994, *supra*). The gel is composed largely of unfermented psyllium polysaccharides (Cabotaje et al., 1994, 1302-1307).

Currently used preparations of psyllium seed husks have certain disadvantages. Laxative preparations of psyllium seed husks are generally composed of ground husk and have coarse and unpleasant mouthfeel when administered in drinks. Psyllium seed husks have been incorporated into cookies, crackers and similar products; however, these products have a tendency to begin to gel unpleasantly in the mouth. More significantly, though, psyllium seed husks can swell in the esophagus, producing an esophageal obstruction that can cause choking. For this reason, psyllium seed husk preparations are not recommended for ingestion by persons who may have difficulty swallowing (e.g., elderly persons). Finally, the recommended daily dose of psyllium husk of 3.5 - 11 g per day is inconvenient to ingest in any form. What is needed is a form of psyllium husk that is convenient and pleasant to use.

Psyllium seed husk has many of the properties of soluble dietary fiber sources. Commonly used sources of soluble dietary fiber include pectin, gums and oat bran. Soluble dietary fiber (SDF) has many uses in food and medicinal preparations. Soluble fibers are components of minimally processed food sources such as oats, oat bran and barley, or are available as concentrates, such as gums, pectins and mucilages. Gums and mucilages are carbohydrate polymers that are generally isolated from plant sources. Mucilages in particular produce slippery or gelatinous solutions in

water. Pectins are polymeric chains of partially methylated galacturonic acids that also possess the ability to form a gel in water. Most soluble fibers are rapidly and completely fermented and have no laxation properties.

Sources of soluble dietary fiber that are also viscous lower serum cholesterol in animals and humans (Marlett, 1997, pp. 109-121, Dietary Fiber and Health, Plenum Press, New York, ed. Kritchevsky and Bonfield). The viscosity of the SDF, rather than its fermentation in the gastrointestinal tract, is key to its hypocholesterolemic action (Marlett et al., 1994, Hepatology 20:1450-1457). Viscosity in the lumen of the lower small intestine interferes with the absorption of bile acids and more bile acids are lost through the stool. Blood cholesterol is thought to be lowered primarily because it is being used in the liver to synthesize more bile acids to replace those lost. The synthesis of bile acids in the liver accounts for 40 to 50% of the daily elimination of cholesterol from the blood. However, the addition of one source of soluble fiber, oat bran, to the diet also increases the proportion of deoxycholic acid in the bile acid pool, which decreases the absorption of exogenous dietary cholesterol. Supplementing the diet with psyllium husk also increases the excretion of bile acids by about 50% (Gelissen et al., 1994, Am. J. Clin. Nutr. 59:395-400).

Soluble dietary fiber concentrates are also often used as thickeners and low calorie fat substitutes in the food industry because of their hydrocolloidal properties (Ward, 1997, Cereal Foods World, 42:386-390). Low-viscosity gums such as gum acacia have both hydrophilic and lipophilic properties that make them ideal as emulsifiers, surfactants and stabilizers. Pectins and mucilages have gel-forming properties that

made them ideal thickeners of food products. Pectins are traditionally extracted from apple and citrus fruits. Commonly used mucilages are generally extracted from seaweed and include carrageenan, agar and alginate. A fat substitute can be made by combining gum with mucilage and/or pectin to create a compound with the emulsifying properties and smoothness of a fat.

SUMMARY OF THE INVENTION

The present invention provides the gel-forming component of psyllium seed husks in a purified form. This gel fraction provides the laxation and hypocholesterolemic effects of intact psyllium seed husks, but is in a form that is easily administrable as a tablet, capsule or liquid, without certain unpleasant or unsafe qualities associated with the use of intact psyllium seed husks. The gel fraction also has utility in treatment of other intestinal abnormalities and maintaining normal bowel function, and as a food thickener and fat replacement.

According to one aspect of the invention, a gel-forming fraction of psyllium seed husks that survives microbial fermentation upon passage through a monogastric mammalian digestive tract is provided. Among other components, the gel fraction comprises predominantly xylose and arabinose in a dry weight ratio of at least about (preferably about 3.5). The fraction comprises notably limited amounts of other sugars, e.g., about 2.5% - 13.5% total of rhamnose, galactose, glucose and uronic acids. More specifically, the gel-forming fraction has the following sugar composition, expressed as a percentage of total sugars:

- between about 0.5% and 4% rhamnose;
- between about 19% and 22% arabinose;
- between about 68% and 76% xylose;

- 5 -

between about 0% and 0.5% mannose;
between about 1% and 2% galactose
between about 0% and 1% glucose; and
between about 1% and 6% uronic acids

5 Upon further purification, the gel-forming fraction becomes even more depleted in rhamnose, glucose and uronic acids.

10 The gel-forming fraction is also highly viscous, a 0.2% concentration in formamide having an apparent viscosity of at least 500 sec, preferably 750 sec, and most preferably 850 sec. The fraction is soluble in a dilute alkaline solution and forms a gel upon acidification of the solution to a final pH of about 4.5.

15 According to another aspect of the invention, in a preferred method of obtaining the psyllium seed husk gel-forming fraction, a separate carbohydrate fraction is also obtained. This fraction is soluble in the dilute alkaline solution and remains soluble upon acidification
20 of the solution to a pH of about 4.5. This fraction is comprised of xylose and arabinose in a ratio of at least about 4:1 and further comprises at least about 12% (by weight) rhamnose and at least about 15% (by weight) uronic acids.

25 According to another aspect of the invention, a method of fractionating psyllium seed husks to obtain a gel-forming fraction and an additional carbohydrate fraction is provided. The method comprises: (a) mixing the husks, in the presence of a chemical reducing agent,
30 in an aqueous alkaline solution comprising between about 0.15 and about 1.0 M (preferably 0.15 - 0.5 M, more preferably 0.15 - 0.4 M, even more preferably 0.15-0.3 M and most preferably 0.15-0.2 M) hydroxyl ions, thereby fractionating the husks into an alkali soluble fraction
35 and an alkali-insoluble fraction; (b) removing the alkali

insoluble fraction; (c) acidifying the alkali soluble fraction to a pH of between about 3 and about 6 (preferably between about 4 and about 5, most preferably about 4.5), which results in the gelation of the gel-forming fraction; and (d) separating the gel fraction, e.g., by centrifugation or other means, from the additional carbohydrate fraction contained in the acidified solution. In preferred embodiments, the method further comprises washing the gel fraction with an aqueous or buffered solution and desiccating the washed gel fraction.

In another aspect of the invention, a gel-forming fraction from psyllium seed husks is provided, which is produced by the aforementioned procedure. The additional carbohydrate fraction is also provided in this aspect of the invention.

According to another aspect of the invention, alternative methods are provided for obtaining a polysaccharide fraction that contains the aforementioned gel-forming fraction. Such a fraction is obtained by solvent extraction using formamide, dimethylsulfoxide or 4-methylmorpholine N-oxide (50% solution in water). The solvent-treated material is centrifuged to recover the soluble materials that are then poured into ethanol to achieve a concentration of 80% ethanol. The precipitate that forms is similar in composition to the alkali-soluble gel-forming fraction.

According to another aspect of the invention, pharmaceutical preparations for treatment of constipation or other intestinal abnormalities, or for lowering serum cholesterol levels in a patient are provided. These preparations are formulated to contain effective dosages of the psyllium seed husk gel-forming fraction of the invention. Methods of treating patients for these various conditions are also provided, which comprise

administering the pharmaceutical preparations of the invention.

Other features and advantages of the present invention will become apparent from the detailed description and examples that follow.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a highly polymerized gel-forming fraction from psyllium seed husks that has great utility in the treatment and prevention of constipation and as a hypocholesterolemic agent. This gel fraction remains substantially unfermented during transit through the gastrointestinal tract (Example 6 and Example 7) and promotes laxation through a variety of means, including increasing moisture content and overall mass of stool and imparting to the stool a slippery characteristic that facilitates ease of passage of stool (Example 6).

The gel-forming fraction from psyllium seed husks has also been demonstrated as a hypocholesterolemic agent. Accordingly, the gel-forming fraction may be used alone or in combination with other active substances as a therapeutic treatment to lower serum cholesterol.

Stool from rats and humans fed psyllium seed husks are sometimes gelatinous (Cabotaje et al., 1994, *supra*; Example 6). However, attempts to purify and characterize a gel-forming material (Kennedy et al., Carbohydrate Res. 75:265-274, 1979; Sandhu et al., Carbohydrate Res. 93:247-259, 1981) have not been successful and none has provided the highly purified gel-forming fraction having the features described in accordance with the present invention. The method of fractionating psyllium seed husks developed in accordance with the invention has led to the unexpected discovery, contrary to published reports (e.g., Kennedy et al.,

1979, *supra*), that a gelatinous, alkali-soluble fraction of psyllium seed husks can be further fractionated to form a highly viscous gel fraction (referred to herein as "Fraction B") and a second carbohydrate fraction with distinctive compositional features, as described in greater detail below (referred to herein as "Fraction C"). Both the viscous, gel-forming Fraction B and the additional Fraction C are soluble in a variety of substances, including dilute and concentrated alkali, formamide, dimethylsulfoxide and 4-methylmorpholine N-oxide (50% aqueous solution); consequently, these two fractions together can be isolated on the basis of these solubility characteristics. However, the further separation of the fractions has been either unsuccessful (e.g., a strong alkali-extracted gel-forming fraction was unable to be further separated by Kennedy et al., 1979; *supra*) or has remained unexplored. The present inventors have discovered that, using a suitable first extraction procedure to obtain a product comprising fractions B and C together, that the fractions can be separated by acidification of a solution containing a mixture of the fractions. Fraction B is concentrated for separation from the acidified mixture by centrifugation, whereas Fraction C remains soluble in the acid.

Description of Carbohydrate Polymers

The viscous, gel-forming psyllium seed husk fraction of the invention (Fraction B) is comprised primarily of xylose and arabinose. In a preferred embodiment, the gel-forming fraction has at least 50% xylose and arabinose by weight, in a more preferred embodiment at least 75% xylose and arabinose by weight, and in a most preferred embodiment at least 85% xylose and arabinose by weight. The gel-forming fraction has an apparent viscosity, as determined in the method of

Example 3, of at least 500 sec, in a more preferred embodiment of at least 750 sec, and in a most preferred embodiment of at least 850 sec. The gel-forming fraction is furthermore particularly deficient in rhamnose, galactose and uronic acids, as compared to xylose. In a preferred embodiment, the ratio of weights of xylose to rhamnose is more than 50, in a more preferred embodiment the ratio is more than 60, and in a most preferred embodiment, the ratio is more than 65. In a preferred embodiment, the ratio of weights of xylose to galactose is more than 25, in a more preferred embodiment the ratio is more than 35, and in a most preferred embodiment, the ratio is more than 42. In a preferred embodiment, the ratio of weights of xylose to uronic acid is more than 15, in a more preferred embodiment the ratio is more than 25, and in a most preferred embodiment, the ratio is more than 35. In a preferred embodiment, the ratio of weights of xylose to arabinose of Fraction B is between 2.5 and 4.5, in a more preferred embodiment the ratio is between 3.0 and 4.0, and in a most preferred embodiment, the ratio is between 3.25 and 3.75.

The acid-soluble psyllium seed husk fraction (Fraction C) is also high in xylose and arabinose. In a preferred embodiment, the acid-soluble fraction has at least 25% xylose and arabinose by weight, in a more preferred embodiment at least 40% xylose and arabinose by weight, and in a most preferred embodiment at least 45% xylose and arabinose by weight. Though Fraction C has an apparent viscosity similar to that of fraction B, it does not have the gel-forming property of Fraction B. Fraction C is furthermore particularly enriched in rhamnose, galactose and uronic acids, as compared to xylose. In a preferred embodiment, the ratio of weights of xylose to rhamnose is less than 6.0, in a more preferred embodiment the ratio is less than 4.5, and in a

most preferred embodiment, the ratio is less than 3.0. In a preferred embodiment, the ratio of weights of xylose to galactose is less than 40, in a more preferred embodiment the ratio is less than 30, and in a most preferred embodiment, the ratio is less than 25. In a preferred embodiment, the ratio of weights of xylose to uronic acid is less than 30, in a more preferred embodiment the ratio is less than 10, and in a most preferred embodiment, the ratio is less than 5.0. In a preferred embodiment, the ratio of weights of xylose to arabinose of Fraction C is more than 3.0, in a more preferred embodiment the ratio is more than 4.0, and in a most preferred embodiment, the ratio is more than 4.5.

Preparation of Psyllium Seed Husk Fractions

The present invention also provides methods of fractionating psyllium seed husks to yield the purified and separated fractions described above. In its most basic form, the method has the following steps:

1. Suspend psyllium seed husks in a dilute alkaline aqueous solution (preferably 0.15 - 0.2 M, hydroxyl ions) containing a reducing agent, in which portions of the husk material will dissolve, while a certain portion remains insoluble;
2. Remove the alkaline-insoluble material (referred to herein as "Fraction A"), e.g., by centrifugation;
3. Acidify the alkali-soluble fraction of step one to a pH of between 3 and 6, preferably 4.5, to yield a gel (Fraction B) and an acid-soluble fraction (Fraction C); and
4. Separate the gel from the acidified solution, e.g., by centrifugation.

One example of this method is taught in Example

1. Many variations exist to the method that will not

substantially change the product isolated. These are described in detail below.

The alkaline solubilization step has several variations. The method taught in Example 1 has improved this solubilization over that found in the prior art. Previous alkaline solubilizations of psyllium seed husk polysaccharides utilized concentrated solutions of base (i.e. 1.2 M NaOH, Kennedy et al., 1979, *supra*) without a reducing agent. Recognizing the harsh nature of this treatment and its partial degradation of polysaccharide chains in the gel-forming fraction, the inventors have demonstrated that a gel-forming fraction can be obtained, presumably in a form more suitable for further fractionation, using a much less concentrated alkaline solution and a suitable chemical reducing agent, such as borohydride. Though up to 4 N alkaline solution can be utilized, the concentration of base in the alkaline solubilization is preferably at least 0.15 N and not more than 1.0 N; in a more preferred embodiment, at least 0.15 N and not more than 0.5 N; and in the most preferred embodiment, at least 0.15 N and not more than 0.2 - 0.3 N. Any standard base can be used in the alkaline extraction, including, but not limited to, sodium hydroxide, potassium hydroxide, lithium hydroxide, ammonium hydroxide and tetramethyl ammonium hydroxide.

A chemical reducing agent, such as borohydride, should be added to the alkaline solubilization step to minimize base-catalyzed depolymerization. In Example 1, a concentration of 1 g/L of sodium borohydride is used, but effective concentrations range from about 50 mg/L to 10 g/L. In a preferred embodiment, the sodium borohydride concentration is at least 100 mg/L and not more than 4 g/L, in a more preferred embodiment at least 500 mg/L and not more than 2 g/L, and in a most preferred embodiment, at least 800 mg/L and not more than 1.2 g/L. Other forms

of borohydride also are suitable for use in this step, including but not limited to, lithium borohydride, potassium borohydride and sodium cyanoborohydride.

The degree of initial processing of psyllium seed husks may alter the alkaline solubilization in ways that will be well known to those skilled in the art. It is important that the husk material be processed so that it is in small pieces, in order to allow the viscous polysaccharides to be easily separated from the insoluble and fibrous materials of the cell walls. In Example 1, the psyllium seed husks are milled, but any process that pulverizes the plant material can be used, and these processes are well known in the art.

The ratio of seed husk material to alkaline solution can be important for efficient solubilization of the polysaccharide fractions. In Example 1, a ratio of 2 g psyllium seed husk is added to 400 ml of alkaline solution, but this ratio can be varied without appreciably affecting the solubilization. For instance, the ratio can be varied so as to add as little as about 0.1 g seed husks or as much as about 4 g seed husks to a 400 ml alkaline solution. Additionally, the time of solubilization can be varied to optimize the procedure (0.5 hr - 24 hr) at a range of temperatures (4-50°C).

Step two of the method of the invention requires that the alkaline insoluble materials be separated from the alkaline soluble materials. In Example 1, centrifugation is employed to accomplish this objective. However, numerous variations and other procedures may be substituted without substantially changing the soluble materials isolated. One skilled in the art will know how to alter the time and force of the centrifugation to adapt the separation to different centrifuge rotors, plant materials and alkaline solutions. Other methods that will accomplish this

separation are well known in the art. Some of these methods will be better suited to large scale use of the method of the invention. Separation methods of interest include, but are not limited to, flow-through centrifugation or filtration (with agitation). Example 1 further teaches washing the insoluble materials with the alkaline solution and re-separation to improve the yield of the alkaline soluble materials. This washing step is optional but can be used to advantage to improve yield.

Step three requires that the alkaline soluble materials of step two be subjected to acidification. In Example 1 this is accomplished by adding glacial acetic acid to the combined alkaline soluble materials until the pH is adjusted to 4.5. The range of pH used for this acid solubilization can be varied without substantial effect on the products. In a preferred embodiment, the pH is between 3 and 6, in a more preferred embodiment, the pH is between 4 and 5, and in a most preferred embodiment, the pH is about 4.5 as described in Example 1. The choice of acid is also subject to variation. Examples of acids suitable for use in this step are acetic, hydrochloric, sulfuric, oxalic, trichloroacetic and trifluoroacetic acids, among others. Here as in step one, the duration, temperature, etc. of the solubilization can be varied, but preferably is carried out at ambient temperature for about 2 hours.

Step four requires that the acid insoluble gel-like material (Fraction B) be separated from the acid soluble materials (Fraction C). Centrifugation is typically employed to accomplish this separation. An optional washing of the insoluble gel mass (e.g., with water, buffer or other suitable solvent) may also be performed to improve the efficiency of the separation.

As alternatives to centrifugation in step four, two other approaches, both of which are amenable to large

scale preparations, may be utilized. The inventors have observed that gel-like material of Fraction B floats, and that the gel has good integrity. Accordingly, the gel can be strained or skimmed from the acidified mixture using a paddle, for instance, such as the type used to remove curds during cheese-making. The gel material is thereafter deposited into a separate container, where it may be washed as a further purification step.

Alternatively, the vessel containing the acidified solution with the gel floating on top can be drained from the bottom by gravity or by gentle vacuum, to leave the gel at the bottom of the vessel. Again, the gel may be washed.

Preparing Fraction B and Fraction C for storage and/or use may employ several procedures. The polysaccharide preparations of Fractions B and C may be used or stored hydrated. If stored in a hydrated form, preservatives or bacteriostatic agents may be added. Drying the polysaccharide preparations is particularly advantageous for use or storage. In a preferred embodiment, Fraction B and Fraction C are desiccated by treatment with 95% ethanol, washing with diethyl ether and drying. The fractions also may be desiccated with other solvents, such as methanol, acetone or isopropyl alcohol. Any standard dehydration method (e.g., evaporation, lyophilization) may be used to dry the fractions, provided the temperature is maintained at less than about 60°C, more preferably at less than about 40°C.

III. Uses of Psyllium Seed Husk Fractions

The psyllium seed husk fractions of the invention have uses as therapeutic treatments. In this regard, the viscous, gel-forming fraction, Fraction B, has been demonstrated effective in promoting laxation and also as a hypocholesterolemic agent. This material can

be used alone or in combination with other active substances in therapeutic or prophylactic preparations for constipation, diarrhea and/or high serum cholesterol. Such preparations can incorporate the gel-forming fraction in pills, capsules or liquids to be administered by mouth. In a preferred embodiment, a dried form of the gel is formulated for convenient administration as a pill or capsule. The gel re-hydrates upon ingestion. With regard to re-hydration, the gel-forming fraction possesses hydration characteristics that are particularly advantageous. After the gel-forming fraction has been isolated and dried, it is slow to hydrate. However, based on observations following feeding the material to colectomized rats, it is clear that the gel does become hydrated in the upper gut, where it exerts its hypocholesterolemic effect. This delay in hydration is advantageous inasmuch as the risk of premature hydration, e.g., by a delay in the esophagus, is minimized or avoided.

The gel-forming preparations can additionally be incorporated into food products. Since the active polysaccharides have been isolated away from the other plant cell components by the method of the invention, they will not have the unpleasant mouthfeel or the necessity to administer large dosages associated with the psyllium seed husk preparations currently in use.

The unfermented gel-forming polysaccharide from psyllium seed husks is well-known for its laxative effects on the monogastric mammalian digestive tract.

For an adult human, a suitable dosage of the gel-forming fraction in dry form is about 2 g, one to three times a day, to maintain bowel regularity and as a treatment for constipation.

As described in Example 4, the gel-forming fraction of psyllium seed husks has been demonstrated as

a hypocholesterolemic agent. Accordingly, this fraction also may be used alone or in combination with other active substances as a therapeutic treatment to lower serum cholesterol. For an adult human, a suitable dosage of Fraction B in dry form is from about 3 g to about 7 g daily. The psyllium husk Fractions B and C of the invention can additionally be used as food additives. They may be used as thickeners, gel-formers and bulking agents in prepared foods. They may also be combined with other food additives to make fat mimetic systems. Because the polysaccharide preparations of the invention are partially non-digested, they will additionally be low calorie, serum cholesterol-lowering and laxation-promoting. The polysaccharide preparations of Fractions B and C can be used in many of the food products where gums and mucilages are currently used.

The following examples are provided to describe the invention in greater detail. They are intended to illustrate, not to limit, the invention.

EXAMPLE 1

Fractionation of Psyllium Seed Husks

5 Milled psyllium seed husks (2 g) were stirred
with 0.2 N potassium hydroxide (400 ml) containing sodium
borohydride (400 mg) in a nitrogen atmosphere for 90
10 minutes. The mixture was centrifuged for 20 minutes at
23,500 x g. The supernatant was decanted from an
insoluble fraction that had settled out in the centrifuge
bottle. The insoluble fraction (designated "Fraction A")
was stirred with fresh potassium hydroxide-sodium
15 borohydride solution (100 ml) for an additional 15
minutes and re-centrifuged. The supernatants from both
centrifugations were combined. The pH of the combined
supernatants was adjusted to 4.5 with glacial acetic
acid, with stirring, at ambient temperature, then
20 centrifuged for 60 minutes at 25,500 x g. The
supernatant (Fraction C) was decanted from a gel mass
(Fraction B) that had settled out in the centrifuge
bottle. The gel was washed gently with water (50 ml) to
remove adhering supernatant solution and this wash water
25 was added to the supernatant. The gel fraction was
desiccated by treatment with 95% ethanol and finally
washed with diethyl ether and dried.

Prior to desiccating Fraction B, in some
instances the fraction was further purified by repeating
30 the alkaline solubilization and acidification steps
described above. The gel was re-suspended in 0.2 N KOH,
acidified to pH 4.5 and centrifuged to recover the gel.

EXAMPLE 2

**Measurement of the Sugar Composition of
Psyllium Seed Husk Fractions B and C**

The respective sugar compositions of the gel fraction (Fraction B) and the acid soluble fraction (Fraction C) produced by the method set forth in Example 1 were determined. Results are shown in Table 1.

Table 1. Composition of psyllium seed husk fractions (dry wt).

	Psyllium Seed Husks (2 g)	Alkali Insoluble (Fraction A)	Alkali Soluble	
			Acid Insoluble (Fraction B)	Acid Soluble (Fraction C)
Yield (g)	--	0.328	1.164	0.255
Composition (% dry wt)				
Rhamnose	3.17	0.49	0.95	14.86
Arabinose	19.93	33.58	19.45	8.11
Xylose	49.15	3.16	67.20	38.79
Mannose	2.17	10.84	0.0	0.0
Galactose	3.83	12.86	1.59	1.89
Glucose	4.37	19.34	0.37	0.75
Uronic Acids	5.41	3.58	1.74	16.84
Ratios of % dry wts				
Xylose/Rhamnose	15.50	6.45	70.70	2.61
Xylose/Arabinose	2.47	0.094	3.46	4.78
Xylose/Galactose	12.83	0.25	42.26	20.52
Xylose/Uronic Acids	9.08	0.88	38.62	2.30

EXAMPLE 3**Measurement of the Viscosity of the
Psyllium Seed Husk Gel-Forming Fraction**

5 Apparent viscosity of the gel-forming fraction
(Fraction B) of psyllium husk was determined. The
solution for apparent viscosity determination was
prepared by stirring overnight a 0.2% solution of the dry
fraction in formamide. The solution was then heated with
10 stirring to 70°C over a 5 minute period, and stirred for
1 hour at ambient temperature. The apparent viscosity
was measured using a pipet viscometer (Ostwald Dropping
Pipet Viscometer, Cat. No. 13-695, Fisher Scientific,
Pittsburgh, PA). The viscosity of the solution was
15 measured three times and mean values determined.

Table 2. Apparent viscosity of psyllium seed husk
Fraction B.

	Apparent Viscosity (sec)
20 Alkaline Soluble, Acid	876
Insoluble Fraction (Fraction B)	
Formamide (solvent only)	303

25

EXAMPLE 4**Solvent Extraction of Psyllium Seed Husk Components**

30 Psyllium seed husk components may also be
obtained by extraction with various solvents. Such
solvents include formamide, dimethylsulfoxide and 4-
methylmorpholine N-oxide (50% solution in water).

Milled psyllium seed husk (2 g) was added in
small portions to a selected solvent (200 ml) with
35 stirring over 30-60 min. In one protocol, the mixture
was stirred for two days at room temperature, then

centrifuged for 40 min at 27,000 x g. In alternative protocols, the mixture was stirred at elevated temperatures, up to 60°C, whereupon the length of stirring could be reduced to as little as 12 hours.

The pelleted insoluble material was resuspended and stirred in additional solvent (50 ml) for 30 min, then re-centrifuged. The combined supernatants were added, with stirring, to 95% ethanol (5 volumes), bringing the final ethanol concentration to 80%.

Methanol, isopropyl alcohol, acetone or similar solvents could be substituted for ethanol in this step. The precipitate was collected and washed with absolute ethanol, followed by diethyl ether, then dried.

The ethanol-insoluble precipitate that forms is similar in composition to the alkali-soluble material described in Examples 1-3. The sugar compositions of fractions obtained by the foregoing initial solvent extraction are shown in Table 3.

Table 3. Sugar compositions of fractions (% dry wt)

	Formamide	4-Methyl- morpholine N-oxide	Dimethyl sulfoxide
Rhamnose	3.03	3.25	0.48
Arabinose	18.95	16.54	17.38
Xylose	65.56	57.47	65.33
Mannose	0.05	0.0	0.12
Galactose	1.54	1.50	1.64
Glucose	0.21	0.22	0.34
Uronic Acids	4.30	4.69	0.94

EXAMPLE 5
Effect of Psyllium Seed Husks and Fractions
Thereof on Reabsorption of Bile Acids from
the Small Intestines of Rats

5 One of the major sites for the
hypcholesterolemic action of dietary fiber is in the
lower small intestine (ileum), through its effect on bile
acids. Bile acids function as emulsifiers in the small
10 intestine to facilitate fat digestion and absorption.
They are synthesized from cholesterol in the liver,
stored in the gall bladder and secreted into the small
intestine in response to eating. Bile acids are
conserved through re-absorption in the ileum and
15 recycling back to the liver through the blood. Food is
digested and fluid absorbed as digesta moves distally in
the intestine, such that in the lower third of the small
bowel, the luminal contents consist largely of
indigestible material (i.e. fiber) and some fluid. It is
20 now known that many of the viscous soluble fiber sources
retain their viscosity during transit through the upper
gastrointestinal tract, and essentially become
concentrated in the lower gut. The soluble fiber
effectively interferes with the re-absorption of bile
25 acids in the ileum, the only site in the gut that has the
necessary transport proteins for bile acids. Bile acids
lost through stool are replaced by new synthesis in liver
from blood cholesterol, which effectively lowers blood
cholesterol levels.

30 Rats were surgically modified by removing the
cecum and colon, and re-connecting the end of the ileum
to the rectum. After a recovery period of 7-10 days,
these animals secrete soft, formed material referred to
as ileal excreta. Ileal excreta is preferred to stool
35 for measuring the effect of a substance on absorption of
bile acids, because bacterial transformation of bile

acids in the large intestine can render up to half of them unidentifiable in stool.

Test meals were fed to groups of rats. Each meal was fed to four rats; the test meals contained 5% fiber as either (1) psyllium seed husk (PSH); (2) cellulose (as a control); or (3) the amount of a selected seed husk fraction (A, B or C), singly or combined, that would be present in 5% psyllium seed husks. The test meals also contained a non-absorbable marker so that the concentration of the marker in the ileal excreta could be used to calculate of the amount of the test meal that was present in the ileal excreta collected.

The results of the acute test meal study are shown in Table 4.

Table 4. Bile acids excreted after feeding test meals containing various soluble fibers.

Test Meal	Bile acids excreted* ($\mu\text{mol/g}$ test meal)
Cellulose	8.32 \pm 2.42
Unmilled PSH	18.41 \pm 2.61
Fraction A	9.07 \pm 2.75
Fraction B	16.08 \pm 3.20
Fraction C	13.76 \pm 2.71
Combined A,B,C	15.65 \pm 1.37
Mean \pm SEM, n = 4 rats	

An increase in bile acids excreted indicates a concomitant decrease in bile acids re-absorbed into the blood. An agent that prevents re-absorption of bile acids into the blood from the ileum is considered to have hypocholesterolemic properties. The results above demonstrate the hypocholesterolemic properties of psyllium seed husks and, more notably, of the conveniently-administrable gel-forming fraction, Fraction B, which was essentially equivalent to intact psyllium seed husks in hypocholesterolemic effect.

EXAMPLE 6

**The Unfermented Gel Component of
Psyllium Seed Husks Promotes
Laxation as a Lubricant in Humans**

5 In addition to increasing stool weight, a
supplement of psyllium seed husks (PSH) produces a stool
that is slick and gelatinous. In this example, we
demonstrate that a gel fraction of psyllium escapes
10 microbial fermentation and is responsible for these
characteristics that enhance laxation.

Materials and Methods:

Experimental design. The study consisted of 3
periods, a screening phase, the PSH period and the basal
15 period. During the screening phase, subjects consumed
15 g/d of Metamucil® (5g/meal) (Smooth Texture Metamucil®,
The Procter & Gamble Co., Cincinnati, OH), along with
their usual diet, for 12 days to provide an additional
8.8 g/d of dietary fiber. Subjects were asked to complete
20 food intake records during d 9-12 to evaluate compliance
with a protocol and to obtain more information about
typical food intakes. Two stools were obtained from each
subject during d 9-12 also to evaluate compliance with
the protocol. During the next 7 days (d 13-19 of the
25 study), subjects consumed a defined, low fiber diet, and
with each meal, the PSH and a nonabsorbable marker
(chromic sesquioxide, 200 mg/meal). For the next two
weeks (d 20-33 of the study), subjects consumed their
usual diet to allow all of the PSH to be excreted. For
30 the 7 days (d 34-40 of the study) of the basal phase, the
same controlled, low fiber diet and nonabsorbable marker,
but no PSH supplement, were consumed. This experimental
design was based on results of a preliminary study. It
was observed in the preliminary study that 7-10 d of
35 ingesting the supplement were necessary to achieve a high
level of PSH excretion. Preliminary observations also

revealed that it took 7-10 d from ingestion of the last test dose for all of the PSH to be excreted. Therefore, the PSH period for data collection followed the 12 d of PSH consumption that constituted the screening phase and a crossover experimental design was not employed.

Stools and qualitative bowel response, food intake and activity data were collected daily during both weeks of controlled diet. This study was approved by the College of Agricultural and Life Sciences Human Subjects Committee, University of Wisconsin-Madison.

Subjects. Twenty-one of the 33 individuals who responded to local advertisements were enrolled in the screening phase. Criteria for exclusion from the screening phase were: lactose intolerance, non-omnivorous diet and unwillingness to follow a specified diet. Subject compliance, reliability, availability and attitude were evaluated during the screening phase, and 15 subjects (8 males and 7 females) were selected to participate in the study. Fourteen subjects completed the study. (Data from one subject who admitted to not providing all stools during the specified collection periods were deleted from the final analysis.) Subjects ranged in age from 18-30 yr (mean \pm SE, 24 ± 1 yr) and were of normal body weight for height (body mass index, 24.2 ± 0.9).

Diet. All subjects consumed fixed diets during the PSH-supplemented and basal phases consisting of foods provided to them as part of the study. Breakfast was consumed at home and consisted of Rice Krispies® cereal, skim milk, orange juice, white bread, margarine or butter, and jelly. Lunch consisted of a sandwich, fresh fruit, and milk. The evening meal consisted of a meat, a starch source, salad and dessert. Subjects consumed prescribed amounts of the menu foods. The amounts of bread and milk varied with daily energy needs. Limited

amounts of fiber-free snacks and alcohol also were permitted. Coffee was allowed *ad libitum*. Subjects were supervised at the evening meal. Consumption of the supplement and nonabsorbable marker was verified daily when empty packets were exchanged for the next day's allotment of supplement.

Data and Sample Collection. All stools excreted during the PSH and basal phases of the study were individually collected, refrigerated promptly, and weighed and frozen within 8 h of collection. Subjects evaluated each stool using a 9 point rating scale consisting of: -4 disagree the most, -3 disagree extremely, -2 disagree very much, -1 disagree, 0 neutral, +1 agree, +2 agree very much, +3 agree extremely, and +4 agree the most possible.

Subjects completed daily food intake records to verify consumption of the items in the controlled diet. Physical activity forms were completed daily to identify any major changes in daily routine. Daily routines for each participant remained consistent throughout the two weeks of sample collection.

Analyses. Stools excreted during d 4-8 of each controlled diet period (d 16-20 and d 37-41 of the study) were thawed, pooled for each subject by hand mixing and re-frozen until use or processed as outlined below. Duplicate aliquots (3 g) were dried (16 h, 70°C) to determine moisture content (Marlett et al., JNCI 76: 1065-1070, 1986). Stool chromium content was determined using a modification (Hosig et al., Cereal Chem. 73: 392-398, 1996) of the method of Guncaga et al. (Clin. Chim. Acta 47: 77-81, 1974). Portions of pooled stool were lyophilized for duplicate (25 mg) determinations of neutral and amino sugar content by gas chromatography as the alditol acetate derivatives after acid hydrolysis and reduction (Kraus et al., J. Chromatog. 513: 71-81, 1990;

Monsma et al., Appl. Environ. Microbiol. 58: 3330-3336, 1992) and for uronic acid analysis using a colorimetric assay (Blumenkranz et al., Anal. Biochem. 54: 484-489, 1973). An aqueous extract of thawed, pooled stool was used to determine the relative viscosity of stool (Ostwald dropping pipet viscometer, Cat. No. 13-695, Fisher Scientific, Pittsburgh, PA). Aqueous fractions were obtained by vortexing aliquots (2 g) with water (10 ml), centrifuging (30,000xg, 30 min, 4°C), and recovering and re-centrifuging the supernatant.

The fractionation procedure to isolate the component responsible for the gel characteristic was applied to thawed aliquots of pooled stool from each subject from both diet periods. Aliquots (25 g) were de-lipidated and subjected to base (0.18 N KOH) containing sodium borohydride (0.026 M) to minimize base-catalyzed depolymerization. The alkali soluble fraction was acidified to pH 4.5 with glacial acetic acid and a precipitate (Fraction 1) recovered by addition to ethanol. Fraction 1 was suspended in water, heated to boiling, centrifuged and the supernatant reduced in volume by roto-evaporation. The concentrate was added to ethanol to give a final alcohol concentration of 70%. The fibrous mass which formed by addition of the concentrated supernatant to ethanol was washed with ethanol, ether and vacuum dried. This material represented the gelatinous component of PSH stools (Fraction 2). No precipitate formed at this point during the analysis of the control stool samples. Neutral and amino sugar contents and uronic acid concentrations were determined on aliquots of this material, as outlined above.

Macronutrient and energy contents of the planned menus and of the daily intakes of each subject were calculated using nutrient composition tables.

Dietary fiber intakes were calculated from food intake records using a detailed database of the fiber content and composition for US foods. The sums of the daily intakes on d 2-6 of each controlled diet period (d 14-18 and d 35-39) were used as the intake of the neutral sugars (glucose, arabinose, xylose, mannose, galactose) and the uronic acids for the determination of apparent digestibility of fiber-derived sugars. The sugar composition of the PSH supplement also was measured by gas chromatography after acid hydrolysis, as outlined above. Fecal excretion of sugars was adjusted to reflect excretion of 5 d of intake using the content of chromium in the pooled stool (Chen et al., Am. J. Clin. Nutr. 68: 711-719, 1998). Apparent digestibilities of fiber-derived sugars were calculated as the difference between intake and excretion, and expressed as a percentage of intake (Chen et al., 1998, *supra*).

Statistical Analyses. Data are reported as the mean \pm SE. Data collected during the basal and psyllium-supplemented phases of the study were compared by one-way analysis of variance using SAS computer program software release 6.12. Significant differences were identified by the least significant difference means separation test.

Results:

The first fraction (Fraction 1) recovered by the gel isolation scheme was extracted from both basal and PSH-containing stools; this alkali-soluble fraction from PSH stools was significantly larger ($P < 0.0005$) than that extracted from basal excreta. An ethanol-precipitable fraction (Fraction 2) that was gelatinous was extracted by hot aqueous treatment of the alkali soluble fraction from stool of subjects consuming the psyllium supplement. No gelatinous fraction was

extracted from stool collected during the basal, low fiber phase of the study.

The major component in Fraction 2 isolated from stool of the 14 subjects consuming PSH was a

5 polysaccharide that contained 763 ± 18 mg of sugar/g of Fraction 2, most of which was xylose (64 ± 1 %) and arabinose (27 ± 0 %); the remainder of the sugars was (%): 2 glucose, 3 galactose, and 3 other sugars (fucose, ribose, mannose, myoinositol, muramic acid, glucosamine and galactosamine). The xylose and arabinose in this gel
10 fraction accounted for 53.9 ± 2.1 and 31.1 ± 1.4 % of the respective sugar in feces.

Since no fraction was obtained from basal excreta comparable to the gel isolated from the
15 PSH-containing stool, aqueous extracts of stool were prepared for a comparison of the relative viscosity of stool from the two study periods. The apparent viscosity of the aqueous extract of the PSH-containing stool was significantly greater ($p < 0.01$) than that of the basal,
20 low fiber excreta, 238 ± 38 vs 128 ± 7 sec.

The PSH supplement increased mean daily wet output from 117 ± 7 to 188 ± 13 g/d ($P < 0.0001$), mean daily dry output from 29 ± 2 to 37 ± 2 g/d ($P < 0.0001$), and stool moisture from 74.4 ± 0.9 % to 80.2 ± 1.0 %
25 ($P < 0.05$). Other measures of large bowel function also were significantly different. The mean wet weight of each stool increased from 121 ± 6 to 173 ± 14 g ($P < 0.005$), mean dry weight of each stool from 30 ± 2 to 34 ± 3 g ($P < 0.0001$), and defecation frequency from $1.0 \pm$
30 0.1 to 1.1 ± 0.1 ($P < 0.05$) when PSH supplement was consumed.

The mixed food controlled diet contained a mixture of fiber-derived sugars and the apparent digestibility of the fiber-derived total neutral sugars
35 was 67 ± 4 % during the low fiber basal period. The

composition of the PSH preparation was (mg/g): 21 rhamnose, 127 arabinose, 325 xylose, 8 mannose, 25 galactose, 44 glucose, 35 uronic acids, 19 ash, 18 crude protein and 346 coating. Apparent digestibilities of the fiber-derived xylose and arabinose decreased ($P < 0.001$), while uronic acid digestibility increased ($P < 0.03$) when the supplement was ingested. Subtracting xylose and arabinose excreted during the basal diet period from the amounts of these sugars in the PSH-containing stool provides a means of estimating the apparent digestibility of the two major sugars in PSH. The apparent digestibilities of the xylose, arabinose and total neutral sugars provided by PSH were 59 ± 5 , 28 ± 7 and $54 \pm 9\%$, respectively and were variable.

The test dose of PSH was well tolerated by all subjects. Compared to large bowel function during the basal period, PSH resulted in gentler bowel movements, softer stools that were easier to pass, greater ease of wiping, a feeling a complete relief, and increased bulk. The PSH supplement had no effect on abdominal cramping, an urge to defecate, or experience a repeat bowel evacuation, nor did it cause diarrhea, although subjects perceived more flatulence and bloating. Although soft and formed, most stools were visibly gelatinous and would vibrate when agitated. The macronutrient and dietary fiber contents provided by the foods in the diet did not change when the PSH supplement was consumed.

Thus, in contrast to other viscous fibers that are completely fermented in the colon, a component of psyllium is not fermented. The results of the foregoing study indicate that, by functioning as an emollient and a lubricant, the unfermented gel isolated from PSH-containing stool represents a new mechanism of laxation for a dietary fiber.

EXAMPLE 7

**Demonstration of Poor Fermentation of the
Gel-Forming Fraction of Psyllium Seed Husks In Vitro**

5 In vitro fermentations of psyllium seed husks
and its fractions were conducted in 40 ml fermentation
flasks. Carbohydrate for fermentation was 200 mg, of
which 31 mg was from veal infusion broth and 45 mg was
from yeast; the remainder was from Fractions A, B and C
10 of psyllium seed husks. Fractions were isolated from
ground psyllium seed husks as described above. The "3
fractions combined" sample consisted of the three
fractions isolated from the psyllium husk re-combined in
proportion to their original concentration in the husks.
15 Inoculum consisted of cecal contents harvested from rats
fed purified diets containing 5% (by weight) psyllium
seed husks. Experimental protocols and analyses were
according to the methods described by Monsma & Marlett
(J. Nutr. 126: 554-563, 1996). Results generated by this
20 methodology have been shown to be similar to results
obtained by *in vivo* fermentation in humans (Monsma et
al., J. Nutr. 130: 585-593, 2000). All fermentations
were prepared and conducted in an anaerobic chamber, and
were performed in duplicate.

25 Table 5 shows the results of the *in vitro*
fermentation. Carbohydrate in the fermentation flasks
consisted of eleven neutral and amino sugars, along with
uronic acids; these were provided by the test substrate
veal infusion broth and yeast (see Table 5 footnotes "a"
30 and "b"). The apparent fermentation of the sugars from
these sources is summarized such that the fermentation of
the test fractions is highlighted. As can be seen from
the table, Fraction C was almost completely fermented by
24 hours. By contrast, Fraction B was only partially
35 fermented (approx. 25-35%), and its fermentation appeared
to have ceased by 48 hours. Fraction A was poorly
fermented, and its fermentation was limited to the first
12 hours of the fermentation period.

Table 5. Disappearance of neutral sugars during *in vitro* fermentation of psyllium seed husk and its fractions

Polysaccharide/sugar	0 h		12 h	24 h	48 h	72 h	
	buffer ^a	fract ^b					
	--mg/flask--		----- % remaining -----				
5	Psyllium husk						
	rhamnose	0.6	4.5	21	22	21	22
	arabinose	0.9	28.1	84	84	76	82
10	xylose	2.6	69.3	68	46	39	40
	galactose	4.3	5.4	61	67	61	66
	others ^c	65.5	9.2	23	22	19	21
	3 fractions combined						
15	rha	0.6	4.4	19	22	22	22
	ara	0.9	29.9	85	81	76	80
	xyl	2.6	70.0	73	47	44	44
	gal	4.3	5.7	56	61	58	60
20	others	65.5	8.6	24	22	21	20
	Fraction C						
	rha	0.6	21.4	16	6	6	6
	ara	0.9	11.6	47	19	18	19
25	xyl	2.6	57.3	53	11	9	8
	uronic acids	3.1	30.5	22	tr	tr	tr
	others	69.9	3.6	14	12	8	9
	Fraction B						
30	rha	0.6	1.3	79	61	52	53
	ara	0.9	26.8	93	85	78	76
	xyl	2.6	91.4	84	76	65	59
	others	69.9	2.2	13	10	10	10
35	Fraction A						
	ara	0.9	49.2	100	100	100	100
	xyl	2.6	4.6	68	58	59	59
	man	15.4	16.0	56	58	65	55
	gal	4.3	19.8	91	89	94	98
40	glc	35.3	29.4	53	50	54	55
	others	17.4	0.7	27	23	19	19

a Sugars in the veal infusion broth and yeast that are part of the fermentate and contributed about 70-75 mg of carbohydrate to the fermentation.

b Sugars in the test substrate, calculated to contribute about 120-125 mg to the fermentation.

c Includes those sugars from the following list that are not specifically listed: rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose, glucose, myoinositol, glucosamine and galactosamine. Although uronic acids were included in the calculation of the amount of carbohydrate to be fermented, they have not been measured in all samples and therefore are not included in this summary, except for the Fraction C summary.

EXAMPLE 8
Reductive Cleavage and NMR Analysis
of Psyllium Seed Husk Fraction B

The polysaccharide of psyllium seed husk Fraction B was methylated fully, then reductively cleaved in the presence of triethylsilane and trimethylsilyl trifluoromethanesulfonate, according to standard methods. The products were acetylated *in situ* and analyzed by gas-liquid chromatography - mass spectrometry (GLC-MS) on a DB-5 column. Results are shown in Table 6.

Table 6. Summary of Reductive Cleavage Analysis

Peak	Residue	Mole Percent
1	Terminal Xylp	23.27
2	Terminal Araf	16.95
3	3-Linked Araf	10.76
4	3-Linked Xylp	4.33
5	Terminal Galp	1.07
6	2,3- or 3,4-Linked Xylp	11.84 ^a
7	2,3-Linked Araf	3.44
8	2,4-Linked Xylp	21.41 ^b
	3,5-Linked Araf	0.58 ^b
9	2,5-Linked Xylf	2.68
10	Unidentified	
11	3-Linked Galp	0.66 ^b
	2,3,4-Linked Xylp	3.01 ^b

^a Standard methylation analysis established that this Xylp residue was 3,4-linked.
^b Coeluting components were separated by GLC on an RTx-200 column.

The components of a reductive cleavage process are more easily separated for identity and quantitation and provide more definitive structural information than those from a standard methylation/hydrolysis procedure.

5 The data set forth in Table 6 indicate that the polysaccharide of Fraction B is a highly branched, very complex molecule. The xylan backbone contains, in addition to single branches of xylose and arabinose, branches of an indeterminate length that may contain both
10 sugars.

The polysaccharide was also subjected to two-dimensional NMR analysis, the results of which indicated that the xylose residues are beta-linked and the arabinose residues are alpha-linked. The latter
15 assignment is supported by the action of alpha-L-arabinofuranosidase on the polysaccharide.

The present invention is not limited to the embodiments described and exemplified above, but is
20 capable of variation and modification without departure from the scope of the appended claims.

We claim:

1. A gel fraction of psyllium seed husks that survives microbial fermentation upon passage through a monogastric mammalian digestive tract, said fraction comprising xylose and arabinose in a dry weight ratio of at least about 3:1 and further comprising less than about 2% (by weight) rhamnose.
2. The gel fraction of claim 1, wherein the xylose to rhamnose dry weight ratio is greater than 50.
3. The gel fraction of claim 1, which comprises galactose, having a xylose to galactose dry weight ratio that is greater than 25.
4. The gel fraction of claim 1, which comprises uronic acids, having a xylose to uronic acids dry weight ratio that is greater than 25.
5. The gel fraction of claim 1, having a sugar composition, based on percent dry weight, of:
 - between about 0 and 3.5% rhamnose;
 - between about 15 and 20% arabinose;
 - between about 55 and 70% xylose;
 - between about 0 and 0.5% mannose;
 - between about 1 and 2% galactose
 - between about 0 and 0.5% glucose; and
 - between about 0.5 and 5% uronic acids.
6. The gel fraction of claim 1, having an apparent viscosity in formamide of at least 500 sec.
7. The gel fraction of claim 1, which is soluble in a dilute alkaline solution and which forms a

gel upon acidification of the solution to a final pH of about 4.5.

8. A pharmaceutical preparation for treatment
5 of constipation in a patient in need of such treatment,
comprising an effective dose of the gel fraction of claim
1.

9. The pharmaceutical preparation of claim 8,
10 wherein the effective dose is between about 2 and about 6
g, based on dry weight, of the gel.

10. A pharmaceutical preparation for treatment
of high blood cholesterol in a patient in need of such
15 treatment, comprising an effective dose of the gel
fraction of claim 1.

11. The pharmaceutical preparation of claim
10, wherein the effective dose is between about 3 and
20 about 7 g, based on dry weight, of the gel.

12. A carbohydrate fraction of psyllium seed
husks, said fraction being soluble in a dilute alkaline
solution and remaining soluble upon acidification of the
25 solution to a pH of about 4.5, said fraction comprising
xylose and arabinose in a ratio of at least about 4:1 and
further comprising at least about 12% (by weight)
rhamnose and at least about 15% (by weight) uronic acid.

13. A method of fractionating psyllium seed
husks to obtain a gel fraction and an additional
carbohydrate fraction, the method comprising:

a) mixing the husks in an aqueous alkaline
solution comprising between about 0.15 and 1.0 M hydroxyl
35 ions, thereby fractionating the husks into an alkali

soluble fraction and an alkali-insoluble fraction;

b) removing the alkali insoluble fraction;

c) acidifying the alkali soluble fraction to a pH of between about 3 and 6, thereby obtaining a gel fraction, and an acidified solution comprising the additional carbohydrate fraction; and

d) separating the gel fraction from the solution containing additional carbohydrate fraction.

10 14. The method of claim 13, wherein the husks are mixed in an aqueous alkaline solution comprising between about 0.15 and 0.3 M hydroxyl ions.

15 15. The method of claim 13, wherein the alkali soluble fraction is acidified to a pH of about 4.5.

20 16. The method of claim 13, which further comprises washing the gel fraction with an aqueous solution.

25 17. The method of claim 16, which further comprises desiccating the washed gel fraction.

30 18. A gel fraction from psyllium seed husks, produced by the method of claim 13.

35 19. A pharmaceutical preparation for treatment of constipation in a patient in need of such treatment, comprising an effective dose of the gel fraction of claim 18.

 20. The pharmaceutical preparation of claim 19, wherein the effective dose is between about 2 and about 6 g, based on dry weight, of the gel.

21. A pharmaceutical preparation for treatment of high blood cholesterol in a patient in need of such treatment, comprising an effective dose of the gel fraction of claim 18.

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22. The pharmaceutical preparation of claim 21, wherein the effective dose is between about 3 and about 7 g, based on dry weight, of the gel.

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23. A carbohydrate fraction from psyllium seed husks, produced by the method of claim 13.

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24. A method of treating constipation in a patient in need of such treatment, which comprises administering to the patient the pharmaceutical preparation of claim 8, in an amount and for a time effective to relieve the constipation.

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25. A method of treating constipation in a patient in need of such treatment, which comprises administering to the patient the pharmaceutical preparation of claim 18, in an amount and for a time effective to relieve the constipation.

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26. A method of lowering serum cholesterol in a patient in need of such treatment, which comprises administering to the patient the pharmaceutical preparation of claim 8, in an amount and for a time effective to lower the patient's serum cholesterol.

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27. A method of lowering serum cholesterol in a patient in need of such treatment, which comprises administering to the patient the pharmaceutical preparation of claim 18, in an amount and for a time effective to lower the patient's serum cholesterol.

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(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/74689 A1

(51) International Patent Classification⁷: A61K 31/715, 35/78

(21) International Application Number: PCT/US00/15693

(22) International Filing Date: 8 June 2000 (08.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/328,611 9 June 1999 (09.06.1999) US

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(81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: UNFERMENTED GEL FRACTION FROM PSYLLIUM SEED HUSKS

(57) Abstract: A purified form of a gel-forming component of psyllium seed husks is disclosed, along with a process for obtaining the gel-forming fraction from psyllium seed husks. Methods of using this gel-forming fraction as a laxative and hypocholesterolemic agent are also disclosed.

WO 00/74689 A1

20000608

DOCKET NO. WARF-0174

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Judith MARLETT and Milton H. FISCHER

International Application No.: PCT/US00/15693

International Filing Date: 08 June 2000

For: UNFERMENTED GEL FRACTION FROM PSYLLIUM SEED HUSKS

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is for the following type of application:

(check one applicable item below)

- | | |
|---|--|
| <input type="checkbox"/> Original | <input type="checkbox"/> Divisional |
| <input type="checkbox"/> Continuation | <input checked="" type="checkbox"/> U.S. National Stage of PCT |
| <input type="checkbox"/> Continuation-in-Part | |

My residence, post office address and citizenship are as stated below next to my name; and I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a

- | | |
|--|--|
| <input checked="" type="checkbox"/> Utility Patent | <input type="checkbox"/> Design Patent |
|--|--|

is sought on the invention, whose title appears above, the specification of which:

- | | |
|--------------------------|---|
| <input type="checkbox"/> | is attached hereto |
| <input type="checkbox"/> | was filed on _____, as U.S. Serial Number _____ |
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☒ was described and claimed in PCT International Application Number PCT/US00/15693, filed on 08 June 2000 and as amended under PCT Article 19 on _____ and/or PCT Article 34 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to the patentability of this application in accordance with 37 CFR § 1.56.

**DISCLOSURE AND/OR CLAIM FOR PRIORITY UNDER
35 U.S.C. §§ 119(a)-(d) OF FOREIGN APPLICATIONS
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I hereby claim foreign priority benefits under Title 35, United States Code §§ 119(a)-(d) of any foreign applications for patent, inventor's certificate or PCT international application designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent, inventor's certificate or any PCT international application designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

Country (or indicate if PCT)	Application Number	Date of Filing (day, month, year)	Priority Claimed Under 37 USC 119a-d
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			<input type="checkbox"/> Yes <input type="checkbox"/> No
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Country (or indicate if PCT)	Application Number	Date of Filing (day, month, year)

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I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date Filed	Status Patented/Pending/Abandoned
09/328,611	June 9, 1999	Patented

**CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
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Provisional Application Number	Filing Date

POWER OF ATTORNEY

I hereby appoint all the practitioners associated with Customer Number 23377 (which is the Customer Number assigned to Woodcock Washburn LLP) to prosecute this application and to transact all business in the U.S. Patent and Trademark Office connected therewith. Each practitioner associated with Customer Number 23377 is an attorney registered before the United States Patent and Trademark Office.

**23377**

PATENT TRADEMARK OFFICE



I hereby appoint the following persons of the firm of **WOODCOCK WASHBURN LLP**, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103 as attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Date: May 2, 2002

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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